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INCREASE OF CYCLIC AMP-DEPENDENT PROTEIN KINASE TYPE II AS AN EARLY EVENT IN HORMONE-DEPENDENT MAMMARY TUMOR REGRESSION

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SUMMARY: Primary, 7,12-dimethylbenz(α)anthracene (DMBA)-induced mammary carcinoma in the rat contains cyclic adenosine 3',5'-monophosphate (cAMP)-dependent and -independent forms of protein kinase. When growth of DMBA-induced tumors was arrested by either ovariectomy or $N^6,O^{2'}$-dibutyryl cAMP treatment of the host, the activity of cAMP-dependent protein kinase type II markedly increased in the tumor cytosol, as shown by DEAE-cellulose chromatography and autophosphorylation. The increase in activity of cAMP-dependent protein kinase was also demonstrable in the tumor cytosol and nuclei following in vitro incubation of tumor slices with cAMP. These results suggest that protein kinase type II is involved in the regression of hormone-dependent mammary tumors.

INTRODUCTION: A number of cyclic adenosine 3',5'-monophosphate (cAMP) effects in eukaryotic systems are known to be mediated through the activation of cAMP-dependent protein kinase(s) (1,2). The cyclic AMP-dependent protein kinases in many tissue extracts can be separated into several fractions by ion exchange chromatography (3-8). Two major fractions have been identified and are referred to as types I and II. The difference between these two types of protein kinase resides in their regulatory subunits (9,10); their catalytic subunits are similar (11,12).

Cyclic AMP arrests whereas estrogen stimulates the growth of 7,12-dimethylbenz(α)anthracene (DMBA)-induced mammary carcinoma in rats (13,14). During the growth arrest of DMBA tumors following either ovariectomy (hormonal deprivation) or $N^6,O^{2'}$-dibutyrylcAMP (DBcAMP) treatment of the host, cAMP-binding and histone kinase activities markedly increased in the tumors (15,16). This study presents evidence that the increase in cAMP-binding and kinase

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activities in regressing tumors can be attributed to the increase in the activity of cAMP-dependent protein kinase type II.

RESULTS AND DISCUSSION: By DEAE-cellulose column chromatography, the cytosols from both growing and regressing DMBA-induced tumors were shown to exhibit two major peaks of protein kinase activity: one stimulated by cAMP (cAMP-dependent) and the other not stimulated by cAMP (cAMP-independent) (Fig. 1). The cAMP-dependent kinase activity in the regressing tumor cytosol was markedly increased and ∿3-fold higher than in the growing tumor cytosol (ovex, Fig. 1). This increase in cAMP-dependent protein kinase activity was further evidenced by a marked increase (∿3 fold) in the cAMP-binding activity of the kinase fraction of the regressing tumor cytosol (Fig. 1). Since the cAMP-dependent kinase peak eluted at high ionic strength (conductivity of 5∿7 m mhos, Fig. 1), the enzyme could be cAMP-dependent protein kinase type II (9). The cAMP-dependent protein kinase type I that elutes at low ionic strength [∿2 m mhos (2)] was not seen in either growing or regressing tumor cytosols (Fig. 1). Fig. 1 also shows that the cAMP-independent protein kinase peak as well as the cAMP-binding peak that is not associated with the protein kinase fraction increased in the regressing tumor cytosol, suggesting an *in vivo* activation of cAMP-dependent protein kinase by cAMP in the regressing tumors following ovariectomy.

By autophosphorylation, i.e., phosphorylation of the regulatory subunit by the catalytic subunit, it is possible to distinguish types I and II of the cAMP-dependent protein kinases in that only type II exhibits autophosphorylation (17,18). This is illustrated in Fig. 2 by the cAMP-dependent protein kinase of DMBA tumor cytosol. The major cAMP-dependent protein kinase peak of bovine heart [type II (17)] also exhibited endogenous phosphorylation (autophosphorylation) by eluting from a DEAE-cellulose column at high ionic strength, whereas the major kinase peak from skeletal muscle [type I (10)] failed to do so (Fig. 2). By using 8-N_3[^{32}P]cAMP, we observed that the regressing DMBA tumor cytosol and nuclei contained the 56,000-dalton regulatory subunit (19,20) of type II protein kinase which co-migrated on SDS-polyacrylamide gels with an

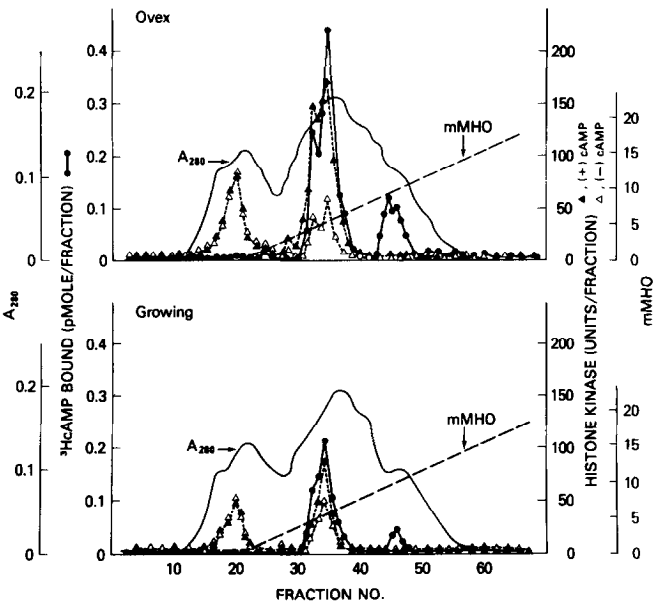

Figure 1. DEAE-cellulose column profiles of protein kinase activity in growing (bottom) and regressing (top) DMBA-induced tumors. Cytosols (105,000 x g supernatant) of growing and regressing (regressed by 20% of the original size at 3 days post ovariectomy) tumors were prepared as previously described (15). Separate but otherwise identical columns (1.0 x 18 cm) were loaded with each cytosol containing 60 mg of protein and eluted at the same flow rate with a common KCl gradient (10-400mM KCl, 10% glycerol in 10mM Tris-HCl, pH 7.0) of 250 ml total volume with a 2.5 ml fraction volume. ————, absorbance at 280; ----, conductivity; protein kinase activity in the absence (Δ) and presence (▲) of 1μM cAMP, using 100 μl of aliquots of corresponding fractions measured as previously described (15). Cyclic AMP binding activity (●) measured with 300 μl of each fraction as previously described (15). The cytosols of growth arrested [3 days post DBcAMP treatment (13)] tumors exhibited a similar elution profile of protein kinase and cAMP-binding activities as regressing tumors of Fig. 1 (top). The rechromatography of the fractions containing protein kinase and cAMP-binding activities exhibited a similar elution profile of the kinase and binding activities as the original column chromatography.

endogenously phosphorylated protein of 56,000-daltons (Y.S. Cho-Chung, M. Schwimmer, and T. Clair, in preparation). Thus our data indicate that DMBA tumor-associated cAMP-dependent protein kinase type II increases during tumor regression induced by hormonal deprivation (ovariectomy) or DBcAMP treatment of the host. This increase in protein kinase type II is an early event rather than the result of tumor regression since the increase in kinase was detectable when there was no appreciable change in tumor size (15). The

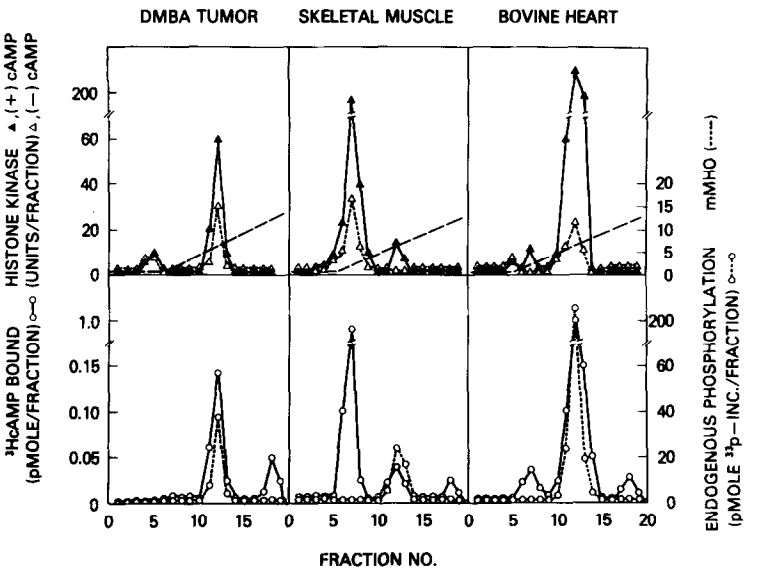

Figure 2. Measurement of histone kinase activity, cAMP-binding activity, and endogenous phosphorylation in fractions obtained from DEAE-cellulose column chromatography. Cytosols prepared from bovine heart, rabbit skeletal muscle, and DMBA tumor (growing) were loaded on to the column and the protein kinases were eluted as described in the legend to Figure 1. Aliquots (100 µl) of the eluates were assayed for histone kinase activity in the absence (△) and presence (▲) of 1 µM cAMP. Other aliquots (200 µl each) were assayed for [^{3}H]cAMP-binding (c——o) and endogenous phosphorylation (o---o), which was done in the absence of both histone and cAMP, and in the presence of 5mM $ZnCl_2$.

early increase in protein kinase activity in the regressing tumor cytosol was followed by a decrease when the nuclei-associated protein kinase reached peak activity. This increase in nuclear kinase activity maybe due to the redistribution of cytoplasmic cAMP-dependent protein kinase into the nucleus (21). Whereas a 1 M KCl extract of the nuclear kinase was not stimulated by cAMP (15), data in Table I show that following the incubation of DMBA tumor slices with cAMP, the nuclear kinase extracted with Triton X-100 was stimulated by cAMP and preferentially utilized histone as an exogenous substrate. These cAMP effects were inhibited when 17β-estradiol was added simultaneously with cAMP to the incubation medium, (Table I). Since the kinase in the control tumor (unincubated) nuclei was not stimulated by cAMP and preferentially utilized casein (Table I), it appears that the cytoplasmic cAMP-dependent protein kinase

Table I

Effects of cAMP and estrogen on the exogenous substrate specificity of protein kinase in DMBA tumor slices in vitro

Preincubation	Substrate	protein kinase activity in Cytosol		Nuclear Extract	
		(-)cAMP	(+)cAMP	(-)cAMP	(+)cAMP
		(n mole/min/mg protein)			
Control	α-casein	0.42	0.39	0.50	0.52
	Histone mixture	0.22	0.45	0.14	0.12
+cAMP	α-casein	0.30	0.30	0.32	0.30
	Histone mixture	0.32	0.90	0.30	0.65
+cAMP	α-casein	0.35	0.35	0.42	0.42
+17β-estradiol	Histone mixture	0.25	0.50	0.20	0.22

Tumor slices were incubated in 5 volumes of buffer A (0.25M sucrose, 2mM $MgCl_2$, 1mM $CaCl_2$, 10mM KCl, 20mM Tris-HCl, pH 7.5) with cAMP (10^{-5}M) in the presence and absence of 17β-estradial (10^{-7}M) at 30°C. Incubations were stopped after 40 min by diluting the incubation mixture with 2.5 volumes of cold buffer A, then tumor slices were immediately centrifuged, and washed once with cold buffer A. The cytosols (105,000 x g supernatant) and purified nuclei were prepared from incubated and unincubated (control) tumor slices as previously described (15). The purified nuclei were suspended in 0.1% Triton X-100 in 10mM Tris-HCl, pH 7.5 (1.0 ml/g nuclear pellet) and extracted at 0° for 2 h. The suspensions were centrifuged at 30,000 x g for 10 min, and the clear supernatants were used as nuclear extracts. Protein kinase assays were performed in the presence and absence of 10^{-6}M cAMP as previously described (15). Values are means of duplicate determinations of 10 pooled tumors.

translocated into the nucleus following the incubation of tumor slices with cAMP.

This study presents the first evidence of a possible role of cAMP-dependent protein kinase type II in the growth regulation of mammary tumors. We have shown that during growth and regression of hormone-dependent mammary tumors, cAMP-binding and cAMP-dependent protein kinase activities are inversely related with estrogen-binding activity (15,16) and cAMP and estrogen elicit opposite effects on specific nuclear protein phosphorylation (22,23). We, therefore, suggest that the interaction of cAMP and steroid hormones in growth

control is expressed through protein kinase(s). Further studies on the various types of protein kinase and their role in the mechanism of action of cAMP and steroid hormones in the growth control of mammary tumors are under investigation.

REFERENCES:

1. Kuo, J.F., and Greengard, P. (1969) Proc. Natl. Acad. Sci. U.S.A. 64, 1349-1355.
2. Krebs, E.G. (1972) Curr Top Cell Regul. 5, 99-133.
3. Reimann, E.M., Walsh, D.A., and Krebs, E.G. (1971) J. Biol. Chem. 246, 1986-1995.
4. Chen, L.J., and Walsh, D.A. (1971) Biochemistry 10, 3614-3621.
5. Yamamura, H., Kumon, A., Nishiyama, K., Takeda, M., and Nishizuka, Y. (1971) Biochem. Biophys. Res. Commun. 45, 1560-1566.
6. Gill, G.N., and Garren, L.D. (1971) Proc. Natl. Acad. Sci. U.S.A. 68, 786-790.
7. Tao, M., and Hackett, P. (1973) J. Biol. Chem. 248, 5324-5332.
8. Beavo, J.A., Bechtel, P.J., and Krebs, E.G. (1974) Methods Enzymol. 38C, 299-308.
9. Rubin, C.S., and Rosen, O.M. (1975) Annu. Rev. Biochem. 44, 831-887.
10. Hoffmann, F., Beavo, J.A., Bechtel, P.J., and Krebs, E.G. (1975) J. Biol. Chem. 250, 7795-7801.
11. Sugdon, P.H., Holladay, L.A., Reimann, E.M., and Corbin, J.D. (1976) Biochem. J. 159, 409-422.
12. Bechtel, P.J., Beavo, J.A., and Krebs, E.G. (1977) J. Biol. Chem. 252, 2691-2697.
13. Cho-Chung, Y.S. (1974) Cancer Res. 34, 3492-3496.
14. Cho-Chung, Y.S., and Gullino, P.M. (1974) Science, 183, 87-88.
15. Cho-Chung, Y.S., Bodwin, J.S., and Clair, T. (1978) Eur. J. Biochem. 86, 51-60.
16. Bodwin, J.S., Clair, T., and Cho-Chung, Y.S. (1978) Cancer Res. 38, 3410-3413.
17. Erlichman, J., Rosenfeld, R., and Rosen, O.M. (1974) J. Biol. Chem. 249, 5000-5003.
18. Rangel-Aldao, R., and Rosen, O.M. (1976) J. Biol. Chem. 251, 3375-3380.
19. Pomerantz, A.H., Rudolph, S.A., Haley, B.E., and Greengard, P. (1975) Biochemistry 14, 3858-3862.
20. Walter, U., Uno, I., Liu, A. Y.-C., and Greengard, P. (1977) J. Biol. Chem. 252, 6494-6500.
21. Cho-Chung, Y.S. (1978) Life Sciences, in press.
22. Cho-Chung, Y.S., and Redler, B.H. (1977) Science 197, 272-275.
23. Cho-Chung, Y.S. (1978) in Endocrine Control in Neoplasia (Sharma, R.K., and Criss, W.E., eds) pp. 335-346, Raven Press, New York.